I concur with this review. M. Serabian 4/11/07

FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Cellular, Tissue and Gene Therapies
Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch

BLA NUMBER: STN #125197.000
DATE PHARM/TOX MODULE
RECEIVED BY CENTER: August 21, 2006
DATE REVIEW COMPLETED: April 11, 2007
PRODUCT: PROVENGE® (Sipuleucel-T or APC8015)
SPONSOR: Dendreon Corporation
PROPOSED INDICATION: Immunotherapy for Asymptomatic Metastatic
                                                  Androgen Independent Prostate Cancer (AIPC)

PHARM/TOX REVIEWER: Yongjie Zhou, M.D., Ph.D.
PHARM/TOX SUPERVISOR: Mercedes Serabian, M.S., DABT
DIVISION DIRECTOR: Ashok Batra, M.D.
OFFICE DIRECTOR: Celia Witten, Ph.D., M.D.
PROJECT MANAGERS: Lori Tull, RAC
                                                  Mark Davidson, B.S., RHIA

Formulation and Chemistry: PROVENGE® consists of autologous peripheral blood mononuclear cells (PBMCs), including antigen presenting cells (APCs) that have been activated *ex vivo* with a recombinant fusion protein, PA2024 that is composed of prostatic acid phosphatase (PAP) and granulocyte-macrophage colony-stimulating factor (GM-CSF). The clinical product is formulated as a suspension in 250 mL of Lactated Ringer's Injection (USP), a physiological solution containing calcium chloride, potassium chloride and sodium lactate, for intravenous (IV) infusion. The color of the suspension will vary between slightly milky to slightly pink, depending upon the amount of white blood cells (WBCs) and red blood cells (RBCs) in the product. The cellular composition of PROVENGE® correlates with the cellular composition of the subject's incoming leukapheresis product. The biologically active components include CD3$^+$ (approximately 55%), CD54$^+$ cells (approximately 25%), CD14$^+$, CD19$^+$ and CD56 $^+$ cells.

Abbreviations: ADT- androgen deprivation therapy; AIPC - androgen independent prostatic adenocarcinoma; APCs **-** antigen presenting cells; b(4) b(4) DCs - dendritic cells; fp-footpads; GM-CSF - granulocyte-macrophage colony-stimulating factor; HLA-human leukocyte antigen; hPAP –human prostatic acid phosphatase; hGM-CSF- human GM-CSF; IL-3-interleukin 3; IHC-immunohistochemistry; IP - intraperitoneal; IV - intravenous; mGM-CSF- murine GM-CSF; MHC-major histocompatibility complex; PAP•GM-CSF- a recombinant fusion protein consisting of PAP protein and GM-CSF protein; PBMCs -peripheral blood mononuclear cells; PSA-prostate-specific antigen; qPCR-quantitative polymerase chain

reaction; rGM-CSF- rat GM-CSF, rPAP-rat PAP; SC-subcutaneous, sp-splenocyte and WBCs-white blood cells

Application History:
BLA rolling submission:
> August 21, 2006
>> • Preclinical section (BLA module 4)
>> • Clinical section (BLA module 5)

> November 9, 2006:
>> • Chemistry, Manufacturing and Controls (BLA module 3)

Cross-references: IND[#] b(4)

Table of Contents

INTRODUCTION

Prostate cancer is the most common, non-dermatologic cancer in men (*1*). In the United States alone, it is estimated that in 2007 approximately 218,890 new cases will be diagnosed and 27,050 men will die of the disease. Generally, a large number of prostate cancers are undetected in normal prostates upon palpation, thus are detected solely on the basis of elevations in serum prostate-specific antigen (PSA). Rarely, subjects present with signs of urinary retention or neurologic symptoms as a result of epidural metastases and cord compression (*2*). Among men with metastatic prostate cancer, >90% have an initial favorable response to primary hormonal therapy with androgen deprivation therapy (ADT) through either surgical or chemical androgen blockade (*3*). However, at later stages, most prostate cancer subjects subsequently progress to an androgen independent state and ultimately relapse due to a refractory response to any form of hormonal therapy. Existing treatment options for men with androgen independent prostate cancer (AIPC)

include second-line hormonal therapy, chemotherapy and investigational agents (*4, 5*), which are intended for palliation and/or prolonging survival. However, currently only Docetaxel in combination with prednisone has been demonstrated to confer a survival benefit (*5*).

The proposed clinical indication (per the sponsor prepared package insert) is for the treatment of men with asymptomatic metastatic AIPC. The label states that PROVENGE[®], an autologous active cellular immunotherapy product, is designed to elicit a specific immune response to a target antigen expressed in prostate cancer (*6-9*).

PROVENGE[®] is a vaccine consisting of autologous PBMCs, which includes APCs that have been activated *ex vivo* with a recombinant fusion protein, PA2024. PA2024 is composed of full-length PAP linked via its COOH terminus to the NH_2 terminus of full length GM-CSF. PAP is a glycoprotein composed of 345 amino acids with a molecular weight of 41 kDa (*10*). PAP, one of the major proteins secreted by prostate columnar epithelium secretory cells following puberty, constitutes approximately 0.5 mg/g wet weight of prostate tissue and is present in seminal fluid at a concentration of approximately 1 mg/ml.   Functionally, PAP is a major phosphatase and a differentiation marker in normal, well-differentiated prostate epithelial cells.   PAP was chosen as the target antigen because while this protein is highly expressed in malignant prostate samples, it is expressed at a much lower level in other normal tissues that have been evaluated. Therefore, the sponsor believes that an immune response against PAP would be an effective means to slow or stop the growth of prostate cancer malignancies.

GM-CSF is a cytokine secreted by activated T lymphocytes, monocytes, macrophages, fibroblasts, endothelial cells, and stromal cells, whose functions are mediated by the binding to a high-affinity receptor (*11*). In humans this receptor is composed of a GM-CSF-specific α chain and a β chain shared by receptors for interleukin 3 (IL-3) and IL-5. The α chain is expressed as a monomer on the plasma membrane of unstimulated cells, including $CD34^+$ progenitor cells, myeloid lineages and vascular endothelial cells. The α chain is responsible for the specific binding of GM-CSF to the receptor. After ligand binding, the β subunit of the receptor is recruited to the α chain/cytokine complex and interacts with the α-bound cytokine to activate signal transduction and functional responses. Thus, GM-CSF predominantly influences leukopoiesis and stimulates the proliferation, differentiation, and activation of granulocytes, macrophages and myeloid-derived dendritic cells (DCs).

The proposed package insert submitted by the sponsor states that PROVENGE[®] is to be administered by intravenous infusion (30-60 minutes) at approximate 2-week intervals, for a total of three infusions. Each dose of PROVENGE[®] is preceded by a standard leukapheresis procedure at approximately 2-3 days prior to the scheduled infusion date. The composition of PROVENGE[®] is dependent on the cells obtained from the subject's leukapheresis. PROVENGE[®] is supplied in an infusion bag labeled for the specific recipient (suspended in 250 mL of Lactated Ringer's Injection, USP) and each dose must be administered to the subject from whom the cells were collected (i.e., autologous therapy).

References:

(1). Jemal A et al. 2007. Cancer statistics 2007. *CA Cancer J Clin*. 57:43-66.
(2). Tarone RE et al. 2000. Implications of stage-specific survival rates in assessing recent declines in prostate cancer mortality rates. *Epidemiology*. *11*:167-170.
(3). Smaletz O et al. 2002. Nomogram for overall survival of patients with progressive metastatic prostate cancer after castration. *J Clin Oncol*. *20*:3972-3982.
(4). So-Rosillo R and Small EJ. 2006. Sipuleucel-T (APC8015) for prostate cancer. *Exp. Rev Anticancer Ther*. *6*:1163-1167.
(5). Dagher R et al. 2004. Approval summary: Docetaxel in combination with prednisone for the treatment of androgen-independent hormone-refractory prostate cancer. *Clin Cancer Res*. *10*:8147-8151.actory prostate cancer. *Clin Cancer Res* 2004; 10:8147-8151.
(6). Small EJ et al. 2000. Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. *J Clin Oncol*. *18*:3894-3903.
(7). Burch PA et al. 2000. Priming tissue-specific cellular immunity in a phase I trial of autologous dendritic cells for prostate cancer. *Clin Cancer Res. 6*:2175-2182.
(8). Burch PA et al. 2004. Immunotherapy (APC8015, Provenge) targeting prostatic acid phosphatase can induce durable remission of metastatic androgen-independent prostate cancer: a Phase 2 trial. *Prostate*. *60*:197-204.
(9). Small EJ et al. 2006. Placebo-Controlled Phase III Trial of Immunologic Therapy with Sipuleucel-T (APC8015) in Patients with Metastatic, Asymptomatic Hormone Refractory Prostate Cancer. *J Clin Oncol*. 24:3089-3094.
(10). Fong L et al. 1997. Induction of tissue-specific autoimmune prostatitis with prostatic acid phosphatase immunization: implications for immunotherapy of prostate cancer. *J Immunol*. 159:3113-3117.
(11). Parmiani G et al. 2006. Opposite immune functions of GM-CSF administered as vaccine adjuvant in cancer patients. *Ann Oncol*. 18(2):226-32.

Preclinical Studies

Due to a species specificity issue with preclinical testing of an autologous human product, the rodent equivalent of Sipuleucel-T was generated. Rodent APCs loaded with fusion proteins composed of either rat PAP (rPAP) fused to rat GM-CSF (rPAP•rGM-CSF) or human PAP (hPAP) fused to murine GM-CSF (hPAP•mGM-CSF) were evaluated in rodents.

Summary of Pharmacology Studies

The following list includes the pharmacology studies that were conducted:

(1). Immunogenicity of hPAP•mGM-CSF Fusion Proteins (Study[#] TR30508)
(2). Generation of PAP Specific, HLA-DR1-Restricted PAP Hybridoma (Study[#] TR30509)
(3). *In vivo* Efficacy Model: Impairment of Tumor Growth by Pre-Immunization with hPAP•hGM-CSF Pulsed Antigen Presenting Cells (Study[#] TR30510)
(4). Induction of Immune Responses to Autologous Protein with PAP Based-Immunization (Study[#] TR30511)

(5). PAP Expression in Human Tissues (Study[#] TR30548)
(6). Induction of Prostate Specific Inflammation in Rats (Study[#] TR30507)

Pharmacology Studies

1. **Immunogenicity of hPAP•mGM-CSF Fusion Proteins**; Study[#] TR30508;
    conducted by Dendreon; non-GLP; 1995

    Species: ▰▰▰ male mice ▰▰▰, 8-10 weeks old (▰▰▰▰▰▰▰▰▰▰▰▰
    ▰▰; the number of mice/group was not provided in the study report

    The purpose of this study was to demonstrate the immunogenicity of hPAP when
    fused to mGM-CSF in a murine model. Based on studies using other self antigens (*1-
    3*) the sponsor hypothesized that evaluation of the immunogenicity of the
    hPAP•mGM-CSF fusion protein antigen when administered *in vivo* would determine:
    (1) whether the mGM-CSF moiety of the hPAP•mGM-CSF fusion protein would
    affect the immunogenicity of hPAP antigen and (2) whether immunological tolerance
    to PAP antigen could be broken. The immunological endpoints for this study
    included anti-PAP specific antibody production and anti-PAP specific T cell
    proliferative responses.

    Study 1 - Humoral response: Animals were immunized intraperitoneally (IP) with
    hPAP or hPAP/▰▰▰▰▰▰▰▰▰▰▰▰▰▰▰▰▰, or immunized with hPAP•mGM-
    CSF fusion protein IP or intravenously (IV) at days 0 and 20 with the same dose of
    protein (Table 1). Blood was collected for assessment of an anti-hPAP antibody
    response on days 12, 20 and 25 after the first immunization. The anti-hPAP antibody
    response was assayed by ▰ b(4) ▰; the amount of serum anti-PAP specific murine IgG
    and IgM was determined by ▰▰ b(4) ▰▰ measurement of the ▰▰▰▰▰▰▰▰
    ▰▰ of each reaction.

    Table 1. Immunization Study 1 Schema: Antibody Response

Test Article	Route	Dose (μg/animal)	Immunization Regimen		Humoral Assay Time Points		
hPAP	IP	40	Day 0	Day 20	Day 12	Day 20	Day 25
hPAP ▰b(4)▰	IP	40					
hPAP•mGM-CSF	IP	220					
hPAP•mGM-CSF	IV	220					

    Results:

    Compared to preimmune sera levels (▰▰▰▰▰▰▰▰▰▰▰▰▰▰▰▰▰), the anti-hPAP
    specific antibody titers were either not detectable or were extremely low at 12 days
    after the first immunization for all groups (▰▰▰▰▰▰▰▰▰▰▰▰▰▰
    However, the day 20 titers for animals immunized IP with hPAP•mGM-CSF were
    comparable to the animals immunized with either hPAP or hPAP formulated in ▰▰
    ▰▰▰▰▰▰▰▰▰▰▰▰▰▰▰▰). The animals immunized IV with

hPAP•mGM-CSF had anti-hPAP-specific titers that were lower ▆▆▆▆ b(4) ▆▆▆▆ ▆▆▆ b(4) ▆▆▆) than the corresponding IP-immunized group (▆▆▆▆ b(4) ▆▆▆▆ ▆▆ b(4) ▆▆ . The day 25 anti-hPAP specific antibody responses for the animals that were IP injected with hPAP•mGM-CSF (▆▆▆▆▆ b(4) ▆▆▆▆▆ were higher than animals receiving hPAP or hPAP/ b(4) (▆▆▆▆▆ b(4) ▆▆▆▆▆ Sera collected on day 25 from the animals immunized IV with hPAP•mGM-CSF was not assayed for anti-hPAP antibodies.

Study 2 - Cellular response: Animals were immunized with hPAP, hPAP b(4) or hPAP•mGM-CSF via IP injection at days 0, 14 and 20 with the same dose of protein (Table 2). Anti-hPAP proliferation responses were determined on day 54 after the first immunization using murine splenocytes from non-immunized animals (normal mice) and from animals immunized IP with hPAP/ b(4) or hPAP•mGM-CSF from the stimulation of the splenocytes *ex vivo* in the presence of hPAP antigen or the control ▆▆▆▆▆ b(4) ▆▆▆▆▆ .

Table 2. Immunization Study 2 Schema: Anti-hPAP Proliferation Response

Test Article	Route	Dose (µg/animal)	Immunization Regimen			Proliferation Assay Time Point
hPAP/ b(4)	IP	50	Day 0	Day 14	Day 20	Day 54
hPAP•mGM-CSF	IP					

Results:

The proliferation response was higher in the hPAP/ b(4) immunized animals (b(4) ▆▆▆▆▆ b(4) ▆▆▆▆▆) compared to the animals immunized with hPAP•mGM-CSF (▆▆▆▆▆ b(4) ▆▆▆▆▆ ▆▆ b(4) ▆▆ . No proliferation response was detected with the splenocytes obtained from normal mice (▆▆▆▆▆ b(4) ▆▆▆▆▆

Study 1 and 2 conclusions: Humoral immune responses to hPAP resulted from immunization of mice with hPAP, hPAP/ b(4) or hPAP•mGM-CSF and cellular immune responses to hPAP resulted from immunization of mice with either hPAP/ b(4) or hPAP•mGM-CSF. Boost immunizations of hPAP, hPAP/ b(4) or hPAP•mGM-CSF administered via the IP route enhanced the humoral response to hPAP in the mice. However, the day 25 anti-hPAP specific antibody titers were higher for the animals that were IP injected with hPAP•mGM-CSF compared to the mice that were IP injected with hPAP or hPAP/ b(4) In addition, the splenocyte proliferation response to hPAP was higher in the hPAP/ b(4) immunized animals compared to the animals immunized with hPAP•mGM-CSF.

Comments:

- The volume of each product administered to the mice was not provided for both Study 1 and Study 2.

- For Study 1, although serum anti-PAP specific murine IgG and IgM antibodies were measured by ███ b(4) ███, the pharmacological activity associated with these titers was not evaluated.
- For Study 2, only the splenocyte proliferation response to hPAP was evaluated for the hPAP█ b(4) █ and hPAP•mGM-CSF immunized animals; CD4$^+$ and CD8$^+$ T cell specific response to hPAP was not assessed.
- A range of dose levels of hPAP and hPAP•mGM-CSF was not used in the studies, thus optimization of dose to cellular and humoral immune response was not determined.

References:

(1). Van Parijs L. and Abbas AK. 1998. Homeostasis and self-tolerance in the immune system: turning lymphocytes off. *Science.* 280:243-248.
(2). Vidovic D et al. 2002. Anti-tumor vaccination with HER-2- derived recombinant antigens. *Int J Cancer.* 102:660-664.
(3). Laus R et al. 2000. Enhanced major histocompatibility complex class I-dependent presentation of antigens modified with cationic and fusogenic peptides. *Nature Biotechnology.* 18:1269-1272.

2. **Generation of PAP Specific, HLA-DR1-Restricted PAP Hybridoma**; Study[#] TR 30509; conducted by Dendreon; non-GLP; 2001-2003

Species: █ b(4) █ mice (█ b(4) █) and ███████ b(4) ███████ transgenic mice (████ b(4) ████ ██████ b(4) ██████); the age, sex and number of mice/group were not provided in the study report. It is assumed that all animals were males.

The purpose of this study was to demonstrate that human PAP can be taken up, processed and presented to a T cell hybridoma in the context of a human HLA-DRβ101 molecule. The transgenic mouse used expresses HLA-DR/H2-E, which is the chimeric MHC class II molecule with HLA-DR-derived peptide-binding of α1 and β1 domains, with a CD4-binding domain derived from mouse I-E sequences ███████████ b(4) ███████████). Thus, the interaction between the endogenous mouse CD4 molecule and its binding epitope on the β2 domain of chimeric class II, which is required for raising the optimal DR-restricted mouse T-cell response, can be evaluated (*1*).

Methods:

To generate human PAP-specific HLA-DR-restricted mouse T-cell lines, ███ b(4) ███ ████ b(4) ████ transgenic mice were immunized subcutaneously (SC) with 20 µg PA2024 (a research-grade recombinant fusion protein, hPAP•hGM-CSF, produced in a█ b(4) █ expression system) in █ b(4) █. One week later mice were sacrificed and lymph node cells were isolated and cultured with 20 µg/mL PA2024 for 3 days, followed by incubation with recombinant human interleukin-2 (rhIL-2) for 3 days. The cultured lymph node cells were then fused to the TCR negative BW5147 T cell

receptor negative thymoma to obtain two IL-2 secreting PAP/DR1-specific CD4$^+$ T-cell hybridoma lines, Papillon and Paperino (*2, 3*).

To demonstrate the antigen specificity and HLA-DR1 restriction of the murine Papillon and Paperino lines, APCs (splenocytes from either wild-type [b(4)] mice or HLA-DR1 transgenic mice) were incubated with $1x10^5$ cells of each hybridoma line in the presence of PA2024 antigen or BA7072 antigen (a portion of human Her2/neu•hGM-CSF). For both hybridomas, IL-2 was produced only when both the HLA-DR1 molecule and PA2024 were present in the culture. Presentation of antigen to the T cell hybridomas was blocked by the addition of an anti-pan HLA-DR antibody, confirming the restriction of the response to the human class II molecule.

To evaluate the ability of these hybridomas to respond to antigen presented by HLA-DR1 positive APCs, three types of HLA-DR1 positive APCs were used to present antigen to the Papillon and Paperino lines: (1) murine splenocytes, (2) murine B cells, and (3) human peripheral blood mononuclear cells (PBMCs). APCs obtained from [b(4)] mice were used as the control. The APCs ($1x10^5$ cells) were incubated with each hybridoma in the presence of PA2024 antigen at concentrations of 0.02, 0.05, 0.1, 0.5, 1.0, 2.0, 4.0, 8.0 10.0, 20, 40, 80, 100, 200 or 400 μg/ml. After a 48-hour incubation, supernatants were harvested and analyzed for IL-2 levels using the IL-2 dependent cell line, HT-2.

Results:

All three APCs presented antigen in a dose dependent manner (Figure 1). The Papillon hybridoma displayed a better response to the murine splenocytes compared to the Paperino hybridoma, but required a higher concentration of PA2024 antigen to respond to the murine B cells and human PBMCs. All three HLA-DR1 positive APCs generated antigen dose dependent production of IL-2 with the Paperino hybridoma line.
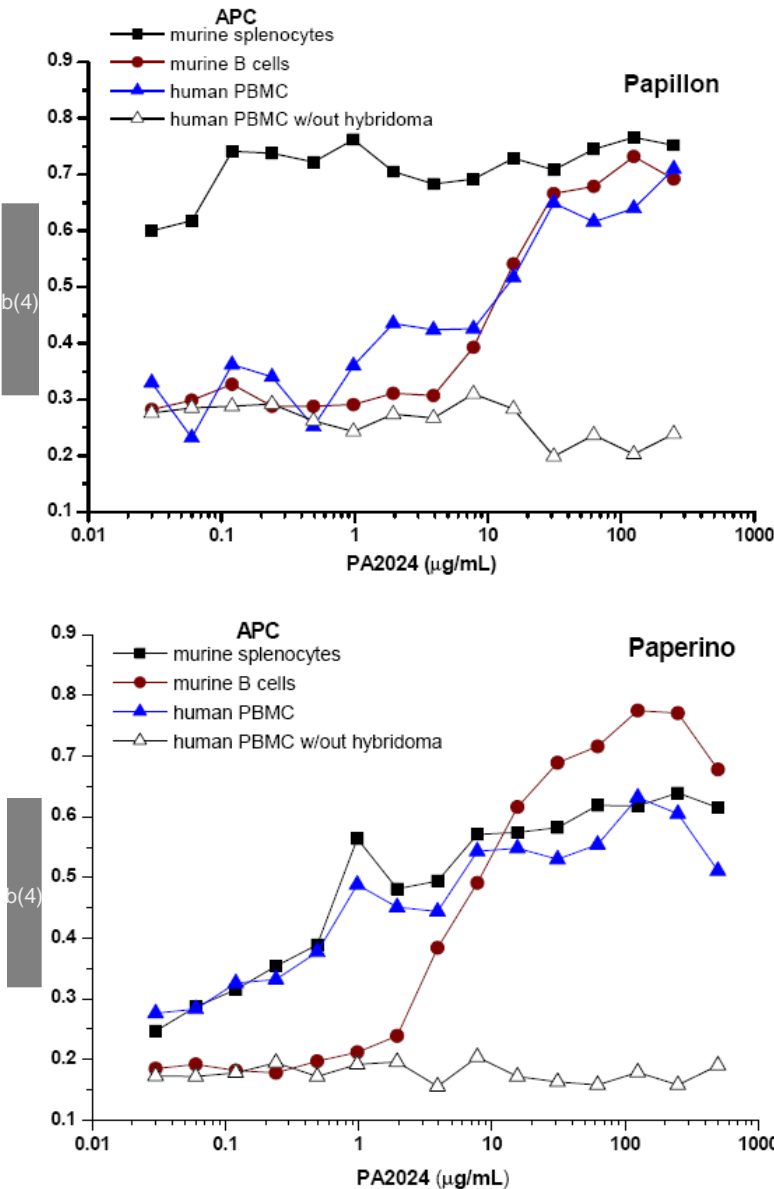
Figure 1. Human and mouse APCs (1×10^5 cells) were incubated with murine Papillon or Paperino hybridomas (1×10^5 cells) in [b(4)]. The APCs (from HLA-DR1^{+} sources) were murine splenocytes, murine B cells, and human PBMCs. PA2024 antigen was added at the indicated concentrations. After a 48-hour incubation, supernatants were harvested and analyzed for IL-2 levels.

The peptide specificity of either the Papillon or Paperino hybridoma was determined

b(4)
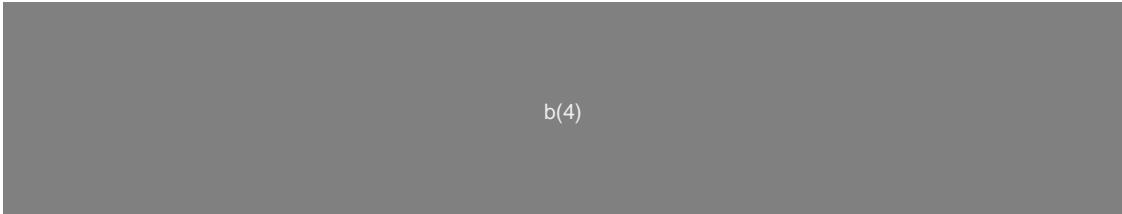
b(4)

Study conclusions: Two murine T cell hybridoma cell lines, Papillon and Paperino, which were PAP specific and HLA-DR1 restricted, were established. The murine hybridomas responded to both murine and human HLA-DR1^{+}APCs. The PAP-specific epitope was mapped to an b(4) amino acid sequence for each hybridoma, suggesting that human PAP can be taken up, processed and presented in the context of a human MHC class II molecule.

References:

(1). Rosloniec EF et al. 1997. An HLA-DR1 Transgene Confers Susceptibility to Collagen-induced Arthritis Elicited with Human Type II Collagen. *J Exp Med.* 185:1113-1122.
(2). Vidovic D et al. 2003. Specific Stimulation of MHC-Transgenic Mouse T cell Hybridomas with Xenogeneic APC. *Human Immunol.* 64: 238-244.
(3). White J et al. 1989. Two Better Cell Lines for Making Hybridomas Expressing Specific T cell Receptors. *J Immunol.* 180:173-181.

3. *In vivo* **Efficacy Model: Impairment of Tumor Growth by Pre-Immunization with hPAP•hGM-CSF Pulsed Antigen Presenting Cells**; Study[#]TR30510; conducted by Dendreon; non-GLP; 2002- 2003

Species: b(4) mice (b(4) ; the age, sex, and number of mice/group were not provided in the study report. It is assumed that all animals were males.

The purpose of this study was to demonstrate *in vivo* anti-tumor immunological activity of hPAP•hGM-CSF fusion protein loaded APCs using a tumor challenge model.

Methods: The cDNAs encoding full length hPAP or human HER2/neu were transfected into the tumorigenic mouse lymphoma cell line EL-4 to generate EL4-hPAP and EL4-hHER2 tumor cell lines (*1-3*). These cells were then used in tumor challenge studies in mice following immunization with hPAP•hGM-CSF-pulsed APCs.   The APCs were derived from adherent b(4) splenocytes cultured with 1 µM hPAP•hGM-CSF antigen b(4) . Mice were immunized IP three times, once every other week, with 2.5×10^5 hPAP•hGM-CSF loaded APCs or APCs alone (controls). Two weeks following the third immunization, the mice were challenged with IP injection of 1×10^5 EL4-hPAP or EL4-hHER2 tumor cells.

Results:

The abnormal clinical observations were not detected in the mice immunized with hPAP•hGM-CSF-pulsed APCs. When challenged with EL4-hPAP tumor cells, these immunized mice survived longer than the non-immunized control mice. All [number of animals not provided] of the non-immunized animals died 3-4 weeks after tumor challenge, while 20% of the immunized mice survived out to days 35-40, with 1 of 10 mice surviving to at least day 60 (study termination). Mice immunized with hPAP•hGM-CSF-pulsed APCs, followed by challenge with EL4-hHER2 tumor cells, showed no survival benefit compared to non-immunized animals.

Study conclusions: Immunization of mice with hPAP•hGM-CSF fusion protein loaded APCs resulted in protective antigen specific immune responses *in vivo*. Following hPAP expressing tumor cell challenge, anti-tumor activity was observed as evidenced by a prolonged survival time in the immunized mice vs. uniform and quick lethality in control (non-immunized or challenged with the tumor cells expressing a different tumor associated antigen, hHER2) mice.

Comments:

- The rationale for the dose level of the hPAP•hGM-CSF loaded APCs, as well as the immunization regimen, was not provided in the study report.
- The immunological endpoints associated with an anti-tumor response mediated by the hPAP•hGM-CSF loaded APCs were not determined.
- Immunization with hPAP•mGM-CSF-pulsed APCs was not evaluated. Both humoral and cellular immune responses to hPAP resulted from immunization of mice with hPAP•mGM-CSF (refer to Study[#]TR30508).
- An established tumor-bearing murine model, which would better mimic the clinical scenario, was not used.

References:

(1). Laus R et al. 2000. Enhanced major histocompatibility complex class I-dependent presentation of antigens modified with cationic and fusogenic peptides. *Nature Biotechnology.* 18:1269-1272.
(2). Vidovic D et al. 2002. Antitumor vaccination with HER-2- derived recombinant antigens. *Int J Cancer.* 102:660-664.
(3). Graddis TJ et al. 2004. Tumor immunotherapy with alternative reading frame peptide antigens. *Immunobiology.* 209:535-544.

4. **Induction of Immune Responses to Autologous Protein with PAP Based-Immunization in Rats;** Study[#] TR30511; conducted by Dendreon; non-GLP; 1996

Species: [b(4)] rats, 2-4 males/group ([b(4)] [b(4)]); the age of the [b(4)] rats was not provided in the study report.

The purpose of this study was to determine whether tolerance could be broken by immunization with PAP derived from the same species (rat) compared to PAP derived from humans.

Methods: Recombinant hPAP or rat PAP (rPAP) proteins and hPAP•mGM-CSF or rPAP•rGM-CSF fusion protein were generated from a ▓▓ b(4) ▓▓ expression system using ▓ b(4) ▓. The rPAP•rGM-CSF fusion protein ▓▓▓ b(4) ▓▓▓ ▓▓▓▓▓▓ b(4) ▓▓▓▓▓▓ Recombinant rPAP ▓▓▓ b(4) ▓▓▓ ▓▓▓▓▓▓ b(4) ▓▓▓▓▓▓ ▓ b(4) ▓ prior to use. Recombinant hPAP and hPAP•mGM-CSF were ▓ b(4) ▓ ▓▓▓ b(4) ▓▓▓

To determine whether exposure to PAP derived from the same species (rPAP) resulted in a qualitatively different immune response from exposure to PAP from another species (i.e., hPAP); rats were immunized with ovalbumin ▓ b(4) ▓ 200 μg/injection), rPAP (200 μg/injection), or hPAP (200 μg/injection), or rPAP plus hPAP (200 μg/injection), followed by measurement of antibody and proliferative T-cell responses. Three experiments were conducted according to the design depicted in Table 1, below. Rats were primed by SC injection of Ova/ b(4) (negative control) or the combinations shown in Table 1 at the base of the tail, followed by boosting on days 8 and 22 with the combinations shown in Table 1, injected into the footpads (fp) or IP. Rats were sacrificed at 10, 14, or 28 days after boost [#]2 (refer to Table 1), serum was collected for measurement of antibody titers (determination of total anti-Ova or anti PAP IgG by b(4) and spleen or draining lymph node cells were harvested and analyzed for T cell proliferation using a ▓▓ b(4) ▓▓ Proliferation assay.

Table 1. Study Designs for the Experiments in Rats

Experiment#1

Rat strain	#/group	Group	Prime (SC)[a]	Int	Boost 1	Int	Boost 2	Sacrifice
b(4)	4	1	Ova b(4)	7d	Ova b(4) (fp)	14d	Ova b(4) (fp)	2/group sac at 14 days; 2/group sac at 28 days
		2	hPAP + rPAP /		hPAP b(4) (fp)		hPAP b(4)(fp)	
		3	b(4)		rPAP b(4) (fp)		rPAP b(4)(fp)	
		4			hPAP•mGM-CSF (IP)		hPAP•mGM-CSF (IP)	
		5			rPAP•rGM-CSF (IP)		rPAP•rGM-CSF (IP)	

Experiment #2

Rat strain	#/group	Group	Prime (SC)	Int	Boost 1 (fp)	Int	Boost 2 (fp)	Sacrifice
b(4)	2	1	Ova/(	7d	Ova/	14d	Ova/ b(4)	10 days
		2	rPAP b(4)		rPAP b(4)		rPAP b(4)	
		3	hPAP		hPAP		hPAP b(4)	
		4	hu+rPAP/ b(4)		hu+rPAP/ b(4)		hu+rPAP/ b(4)	

Experiment#3

Rat strain	#/group	Group	Prime (SC)	Int	Boost 1 (fp)	Int	Boost 2 (fp)	Sacrifice
b(4)	2	1	Ova/	7d	Ova/	14d	Ova b(4)	10 days
		2	rPAP b(4)		rPAP b(4)		rPAP/ b(4)	
		3	hPAP		hPAP		hPAP/ b(4)	
		4	hu+rPAP/ b(4)		hu+rPAP b(4)		hu+rPAP/ b(4)	

a. Abbreviatons: SC, subcutaneous; fp, footpad; IP, intraperitoneal; Int, interval between injections

Results:

Antibody response: Sera from Ova/ b(4) immunized rats showed reactivity to Ova stimulation, but no reactivity to the different PAP protein preparations (Figure 1). Rats immunized with rPAP, hPAP, or the combination displayed a high level of IgG antibody titers (b(4)) for both recombinant PAP proteins used for immunization (Figure 2). Cross-reactivity to rPAP was detected in sera from animals immunized with hPAP (the sequence homology between the rPAP and hPAP mature proteins≈ 79.9%). In addition, sera from all rats immunized with the different forms of PAP reacted with b(4) derived hPAP, while those rats immunized with Ova did not (Figure 3).
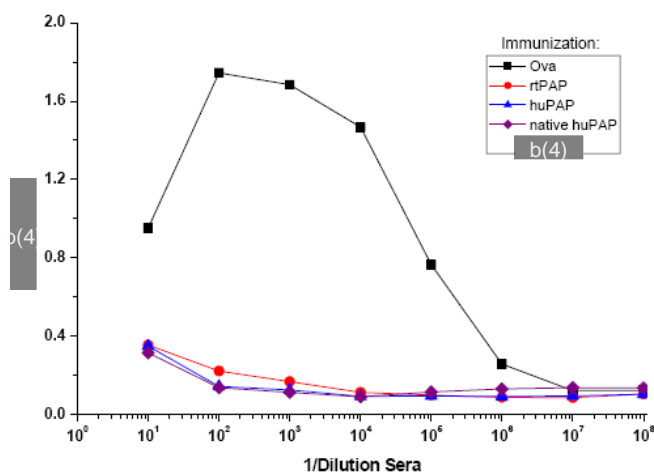

Figure 1: Antibody responses in Ova/ b(4) immunized rats, as determined by b(4) The serum from each rat was analyzed; the mean data are presented (testing conducted three times).
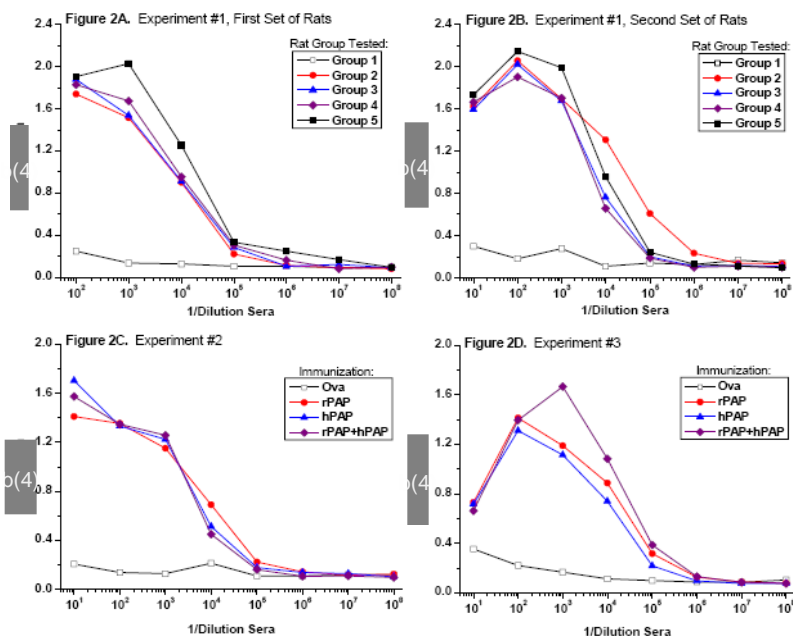
Figure 2. Antibody responses to rPAP, as determined by [b(4)] were [b(4)] with recombinant rPAP. The serum from each immunized rat was analyzed; the mean data are presented.
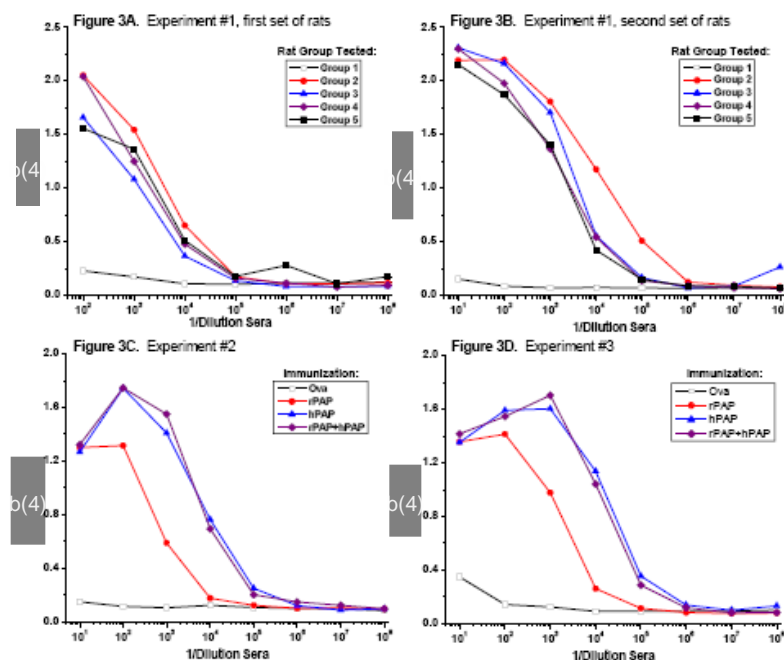

Figure 3: Recombinant hPAP-specific antibody titers, as determined by [b(4)] with recombinant [b(4)] hPAP. The serum from each immunized rat was analyzed; the mean data are presented.

Cellular response: Lymph node cells (LNCs) isolated from rats immunized with Ova antigen proliferated in response to Ova, but not in response to PAP proteins derived from recombinant [b(4)] or from natural sources (Figure 4). Lymph node cells from rats immunized with rPAP showed a dose dependent response towards rPAP (Figure 4B). A response to recombinant hPAP was seen in some rats (Figure 4A), but was more variable between experiments (Figure 4B and 4C). Data from a separate study showed that CD4$^+$, but not CD8$^+$ T cells, isolated from hPAP•mGM-CSF immunized mice, mediated significant inhibition of tumor cell growth *in vitro* (*1*).

4A

4B

4C

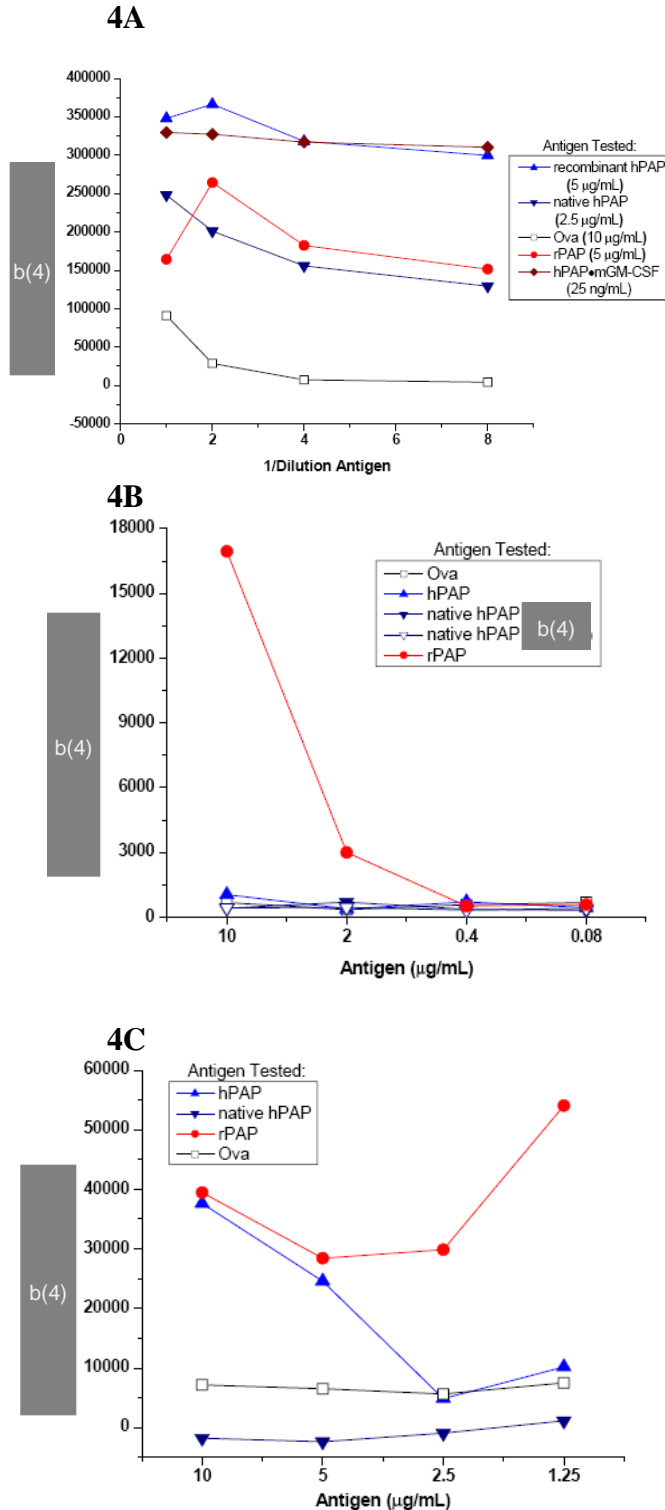**Figure 4.** Proliferative responses of rat lymph node cells (LNCs) *in vitro*. Rats were previously immunized and boosted with recombinant rPAP, according to Table 1 (**A**). Rat LNCs from Experiment #1: Group 3= hPAP + rPAP/ b(4) prime; rPAP/b(4) boost; (**B**). Rat LNCs from Experiment #3: Group 2 = rPAP/ b(4) prime; rPAP/b(4) boost; (**C**). Rat LNCs from Experiment #2: Group 2 = rPAP/ b(4) prime; rPAP/b(4) boost.

Study conclusions: Immunizing rats with recombinant rPAP and hPAP induced high level IgG antibody titers. These data suggest that, in this animal model system, PAP immunization has the ability to bypass B cell immune tolerance to a prostate specific antigen. The proliferation of splenocytes in response to recombinant rPAP and recombinant hPAP was observed. The CD4^{+} T cells purified from hPAP•mGM-CSF-immunized animals mediated significant inhibition of tumor cell growth.

Comments:

- The sponsor did not provide the rationale to support the dose levels for the various agents that were used in this study. In addition, the dose volume of each agent injected was not specified.
- The sponsor did not evaluate the relationship between the humoral and/or cellular-mediated anti-PAP specific immune response seen in the animals with anti-tumor activity.

References:

(1). Laus R et al. 2001. Dendritic cell immunotherapy of prostate cancer: preclinical models and earlier clinical experience. *Cancer Research Therapy and Control.* 11:1-10.

5. **PAP Expression in Human Tissues**; Study [#]TR30548; b(4) conducted by b(4) b(4) (non-GLP); b(4) Conducted by Dendreon (non-GLP); b(4) Analysis of PAP mRNA Expression Conducted by Dendreon (non-GLP); 1999-2004

The sponsor provided information regarding the tissue specificity of PAP expression in normal and malignant human tissues from: (1) a summary of the published literature on PAP expression in malignant non-prostate tissues (Table 1) and in normal non-prostate tissues (no table provided) and (2) data collected from studies conducted by Dendreon (Tables 2-4).

Methods:

Published data: PAP expression in normal and malignant tissues was determined by immunohistochemistry (IHC) staining, quantitative polymerase chain reaction (qPCR), or Northern-Blot hybridization.

Dendreon-generated data: PAP expression in normal and malignant prostate tissues was determined by the following methods: (1) b(4) b(4) by b(4)) b(4) PAP- b(4)

b(4)

PAP mRNA distribution in the human tissues conducted with the [b(4)] [b(4)]) and the [b(4)] [b(4)] which was derived from the data bases at the National Cancer Institute Cancer Genome Anatomy project resource (NCBI accession number NM_001099 and cluster Hs. 433060).

Results:

Information obtained from the published scientific literature: PAP expression was detected in >95% of the primary prostate adenocarcinomas evaluated. PAP expression was also detected in other tumors of non-prostatic origin, such as colorectal carcinoids, islet cell tumors, ovarian adenocarcinomas, metastatic breast carcinoma, bladder tumors with mixed glandular and transitional features, adenomas of the salivary gland, and lung carcinosarcoma (Table 1). In addition, PAP expression, determined via non-quantitative analysis, was detected in normal non-prostate tissues, such as pancreatic islet cells, parietal cells of the stomach, renal tubular epithelial cells and loops of Henle, distal tubules in fetal and adult kidneys, liver cells, urethral glands of both sexes, renal maculae densae, salivary glands, and rectal tissue, at levels that are notably lower than in normal and cancerous prostate tissue and in other non-prostate tumors (*1-18*).

Table 1. PAP Expression in Malignant Non-Prostate Tissues as Determined by IHC (from the published literature)

Tissue	No. PAP+/ No. tested	Reference
colon tumor	5/5	Wang 2005
gastric tumor	5/7	Wang 2005
breast tumor	2/5	Wang 2005
ovarian adenocarcinoma	1/55	Haines 1989
colorectal carcinoid	1/2	Haines 1989
urinary bladder adenocarcinomas (11 male, 4 female)	5/15	Epstein 1986
bladder tumors with mixed glandular and transitional features (5 male, 4 female)	3/9	Epstein 1986
proliferative lesions of the bladder	10/34	Nowels 1988
intravascular large B cell lymphoma	5/5	Seki 2004
rectal carcinoid	23/28	Kimura 1986
appendical carcinoid	2/3	Kimura 1986
	1/7	Haines 1989
small intestine carcinoid	2/7	Kimura 1986
	4/12	Haines 1989
stomach carcinoid	1/6	Kimura 1986
kidney carcinoid	1/1	Kimura 1986
ovarian carcinoid	1/1	Kimura 1986
jejunum and ileum carcinoids	10/51	Burke 1997
gastrointestinal carcinoids (non-rectal)	12/81	Sobin 1986
hindgut rectal carcinoid	16/24	Sobin 1986
carcinoid tumors of various origins	0/27	Cohen 1983
rectal and colon carcinoids	62/76	Federspiel 1990
islet cell tumor of the pancreas	2/8	Cohen 1983
	1/1	Choe 1978
	1/9	Haines 1989
adenocarcinoma of the paraurethral gland and ducts	1/1	Zaviacic 1993
salivary duct carcinoma	7/12	Fan 2000
neuroendocrine tumor of the pancreas	1/1	Kaneko 1995
	6/10	Jobsis 1981

<u>Data generated by Dendreon:</u> Assessment of ▮▮▮ b(4) ▮▮▮ analysis, and ▮▮▮ b(4) ▮▮▮ Analysis of PAP tissue showed that PAP is expressed at high levels in both normal and malignant prostate tissue and is also expressed at substantially lower levels in some normal non-prostate tissues. Assessment by ▮b(4)▮ showed PAP expression in normal breast, colon, pancreatic islet cells, prostate and skin (Table 2). The normal tissue distribution of PAP mRNA by ▮b(4)▮ showed prostate > bladder > kidney > pancreas > cervix/testes > lung/ovary (Table 3). The quantitative data from the ▮▮▮ b(4) ▮▮▮ analysis suggested a hierarchical tissue distribution of PAP mRNA expression, based on the expression level of the PAP gene (Table 4).

Table 2. b(4) Study of PAP Protein Expression in Normal and Malignant Human Tissues

Frequency of PAP+ Signals				
	with mouse anti-PAP mAb		with rabbit anti-PAP pAb	
Tissue	Malignant	Normal	Malignant	Normal
Breast	0/10	2/2 epithelium only	0/10	0/2
Colon	3/10	2/2 rare neuroendocrine cells	3 [a]/10	2/2 rare neuroendocrine cells
Lung	2/10	0/2	2 [a]/10	0/2
Ovary	2/10	0/2	2/10	0/2
Pancreas	1/10 islet cells only	2/2 islet cells only	2/10 islet cells only	2/2 islet cells only
Prostate	10/10	2/2	10/10	2/2
Skin	Not Done	2/2	Not Done	2/2

[a] Only rare lymphocytes stained positive in one positive sample.

Table 3. b(4) Study of PAP mRNA Expression in Normal and Malignant Human Tissues

Tissue	Normal		Tumor	
	mRNA Expression Value	% of Normal Prostate	mRNA Expression Value	% of Normal Prostate
Breast	4	0.07	7	0.13
Bladder	115	2.08	12	0.22
Cervix	12	0.22	116	2.09
Colon	4	0.07	6	0.11
Kidney	31	0.56	11	0.19
Liver	1	0.02	2	0.04
Lung	8	0.14	24	0.43
Ovary	8	0.14	22	0.39
Pancreas	16	0.28	5	0.09
Prostate	5525	100.00	13264	240.07
Testes	12	0.22	11	0.19

Table 4. [b(4)] Analysis [b(4)]) of PAP mRNA Expression in Normal and Malignant Human Tissues

Tissue	b(4) Data Normal	Cancer	b(4) Data Normal	Cancer	b(4) Data Normal	Cancer	P	b(4) Data Normal	Cancer	P
ALL TISSUES					381 / 3430002	247 / 2534701	0.05	68 / 7867893	118 / 11786314	0.17
adrenal cortex	--		--	--	--	0 / 10599	--	--	--	--
adrenal medulla	--		--	--	--	1 / 634	--	--	--	--
Bone			--		0 / 13791	4 / 59604	0.30	--	0 / 83577	--
bone marrow					0 / 15915	2 / 29383	0.32	0 / 204563	0 / 78327	--
Brain					0 / 349131	4 / 208561	0.07	0 / 729526	0 / 4662114	--
cartilage			--		0 / 14373	1 / 37784	0.42	--	4 / 778035	--
cerebellum					0 / 85696	0 / 0	--	0 / 90885	0 / 1495938	--
cerebrum			--	--	0 / 148158	0 / 3414	--	--	--	--
Cervix			--	--	0 / 3607	0 / 44937	--	--	--	--
Colon					0 / 28484	5 / 174457	0.31	0 / 98089	0 / 643586	--
Ear			--	--	0 / 16942	--	--	--	--	--
embryonic tissue			--	--	1 / 198148	--	--	--	--	--
endocrine			--	--	0 / 16882	0 / 3315	--	--	--	--
esophagus			--	--	0 / 83	0 / 16635	--	--	--	--
Eye				--	3 / 123375	0 / 47258	0.29	0 / 85898	--	--
gastrointestinal tract				--	0 / 28151	0 / 13155	--	0 / 45908	--	--
genitourinary			--	--	0 / 9043	0 / 27711	--	--	--	--
head and neck			--	--	2 / 45844	7 / 104759	0.34	--	--	--
Heart		--		--	3 / 77038	--	--	0 / 83063	--	--
Kidney					1 / 107426	0 / 92702	0.37	1 / 106467	0 / 100281	0.37
Limb	--	--	--	--	--	--	--	--	--	--
Liver					1 / 107494	0 / 90183	0.37	0 / 66308	1 / 214987	0.43
Lung					0 / 135523	0 / 181875	--	0 / 159917	0 / 159059	--
lymph node					0 / 68957	0 / 27746	--	0 / 99426	2 / 72008	0.22
lymphoreticular			--	--	0 / 18183	0 / 44914	--	--	--	--
mammary gland					0 / 56550	41 / 118233	0.00*	0 / 509556	0 / 1717147	--
Muscle					22 / 82478	0 / 28330	0.01	0 / 107836	0 / 61270	--
nervous			--	--	0 / 223109	0 / 0	--	--	--	--
Ovary					1 / 11965	0 / 96252	0.26	0 / 94887	0 / 179472	--
pancreas					0 / 7493	0 / 75948	--	0 / 85728	0 / 189999	--
Pancreatic islet			--	--	6 / 101401	0 / 30918	0.19	--	--	--
parathyroid	--		--	--	--	0 / 21158	--	--	--	--
peripheral nervous system			--	--	7 / 15935	0 / 927	0.38	--	--	--
pineal gland		--	--	--	0 / 7052	--	--	--	--	--
pituitary gland			--	--	0 / 14108	0 / 1616	--	--	--	--
placenta				--	2 / 261277	1 / 40242	0.35	0 / 207348	--	--
pooled tissue			--	--	1 / 298862	0 / 0	--	--	--	--
prostate					329 / 85909	170 / 69431	0.00*	62 / 266949	111 / 491794	0.44

* P < 0.00001.

Study conclusions: The PAP protein and gene are highly expressed in both normal and malignant prostate tissue, as well as in a limited set of non-prostate normal tissues; however, the level of PAP protein or gene expression in these non-prostate tissues appears to be notably lower than the levels found in normal and malignant prostate tissues.

References:

(1). Wang Y et al. 2005. Prostatic Acid Phosphatase as a Target Molecule in Specific Immunotherapy for Subjects With Non-prostate Adenocarcinoma. *J Immunother*. 28:535–541.

(2). Haines AMR et al. 1989. A Novel Hybridoma Antibody (PASE/4LJ) to Human Prostatic Acid Phaosphatase Suitable for Immuneohistochemistry. *Br J. Cancer.* 60:887-92.

(3). Epstein JI et al. 1986. Prostatic Acid Phosphatase Immunoreactivity in Adenocarcinomas of the Urinary Bladder. *Human Pathology.* 17:939-942.

(4). Nowels K et al. 1988. Prostatic Specific Antigen and Acid Phosphatase-Reactive Cells in Cystitis Cystica and Glandularis. *Arch Pathol Lab Med.* 112:734-737.

(5). Seki K et al. 2004. Prostatic Acid Phosphatase Is a Possible Tumor Marker for Intravascular Large B-Cell Lymphoma. *Am J Surg Pathol.* 28:1384–1388.

(6). Kimura N et al. 1986. Prostatic Specific Acid Phosphatase in Carcinoid tumors. Virchows Arch A Pathol Anat. *Histopath.* 410:247-251.

(7). Burke AP. 1997. Carcinoids of the Jejunum and Ileum. *Cancer.* 79:1086-1093.

(8). Sobin LH et al. 1986. Prostatic Acid Phosphatase Activity in Carcinoid Tumors. *Cancer.* 58:136-138.

(9). Cohen C et al. 1983. Prostatic Acid Phosphatase in Carcinoid and Islet cell Tumors. *Arch Pathol Lab Med.* 107:277.

(10). Choe BK et al. 1978. Expression of Human Prostatic Acid Phosphatase in A Pancreatic Islet Cell Carcinoma. *Invest Urol.* 15:312-318.

(11). Zaviacic M et al. 1993. Prostatic Specific Antigen and Prostate Specific Acid Phosphatase in adenocarcinoma of Skene's Paraurethral Glands and Ducts. *Virchows Arch A Pathol Anat. Histopath.* 423:503-505.

(12). Fan CY et al. 2000. Expression of Androgen Receptor and Prostatic Specific Markers in Salivary Duct Carcinoma. *The American J Surg. Pathol.* 24:579-586.

(13). Kaneko Y et al. 1995. Neuroendocrine Tumors of the Liver and Pancreas Associated with Elevated Serum Prostatic Acid Phosphatase. *Intern Med.* 34:886-891.

(14). Jobsis AC et al. 1981. The Immunohistochemical Detection of Prostatic Acid Phosphatase: Its Possibilities and Limitations in Tumor Histochemistry. *Histochem J.* 13:961-73.

(15). Li YC et al. 1980. Immunohistochemical Diagnosis of Prostatic Cancer with Metastasis. *Cancer Lett.* 46:706-712.

(16). Kamoshida S et al. 1990. Extraprostatic Localization of Prostatic Acid Phosphatase and Prostatic-Specific Antigen: Distribution in Cloacogenic Glandular Epithelium and Sex-Dependent Expression in Human Anal Gland. *Human Pathol.* 21:1108-1111.

(17). Elgamal A et al. 1996. Detection of Prostatic-Specific Antigen in Pancreas and Salivary Glands: A potential Impact on Prostate Cancer Overestimation. *J Urol.* 156:464-468.

(18). Cunha AC et al. 2005. Tissue-specificity of prostate specific antigens: Comparative analysis of transcript levels in prostate and non-prostatic tissues. *Cancer Lett.* 236:229-238.

6. Induction of Prostate Specific Inflammation in Rats; Study[#] TR30507; conducted by Dendreon; non-GLP; 1995-1997

Species: [b(4)] rats ([b(4)] Exp[#]1) or [b(4)] Exp[#]2 or from [b(4)] Exp[#]3), 10 weeks old, 4-10 males/group

The purpose of this study was to assess the induction of prostate-specific immunity in rats following PAP antigen immunization (*1*).

Experiment [#]1: [b(4)] rats were immunized SC (prime) with Ova, hPAP, or rPAP on day 0, followed by boost (on days 7 and 21; injected in the foot pad [fp] or IP) with either Ova or a derivative of PAP (hPAP, rPAP, rPAP•rGM-CSF, or hPAP•mGM-CSF), [b(4)] (Table 1).
.

The PAP protein prime and boost schedule was designed to determine if PAP-specific immune tolerance could be broken *in vivo* (*2-3*). On day 35 (two weeks after the final boost), 2 rats/group were sacrificed and blood and tissues collected for *ex vivo* T cell proliferation and antibody assays. The prostate was harvested from the remaining 2 rats/group and microscopically examined for potential cross-reactive autoimmunity (analysis by [b(4)]

Table 1. Experiment [#]1: Protein prime and protein boost in [b(4)] male rats

Group	Rats	Prime (day 0)	Boost (day 7 and day 21)
1	4	20 µg Ova/[b(4)] (SC)	Ova [b(4)] (fp)
2	4	7.5 µg hPAP + 10 µg rPAP/[b(4)] (SC)	hPAP [b(4)] (fp)
3	4	7.5 µg hPAP + 10 µg rPAP/[b(4)] (SC)	rPAP/[b(4)] (fp)
4	4	7.5 µg hPAP + 10 µg rPAP/[b(4)] (SC)	hPAP•mGM-CSF (IP)
5	4	7.5 µg hPAP + 10 µg rPAP/[b(4)] (SC)	rPAP•rGM-CSF (IP)

Results:

There were no significant microscopic changes in the prostates for Groups 1-3. Boosting with the hPAP•GM-CSF-fusion protein resulted in induction of mild prostate inflammation (Groups 4-5). One out of two animals boosted with hPAP•mGM-CSF (Group 4) developed a grade 1 (minimal) multifocal lymphocytic interstitial inflammation of the prostate. Two out of two animals boosted with rPAP•rGM-CSF (Group 5) developed a grade 1 multifocal lymphocytic interstitial inflammation of the prostate, suggesting that PAP•GM-CSF fusion proteins, not PAP, are capable of breaking immune tolerance in the prostate.

Experiment [#]2: The cellular immunizations with (syngeneic antigen-naïve rat spleens (spDCs) that were pulsed overnight with rPAP•rGM-CSF fusion protein (0.2 mg/ml) were designed to mimic the human studies (Table 2). [b(4)] male rats were immunized (prime) with 1.56×10^7 enriched DCs/rat on day 0, followed by boost [#]2 with 9.5×10^6 cells/rat (day 14) and boost [#]3 with 6.2×10^6 cells/rat (day 28). Un-immunized syngeneic rats (age- and sex-matched) were used as controls. On day 42 (2 weeks after the final boost) four rats from the control (C) and four rats from the treated (T) group were sacrificed and brain, lung, heart, liver, kidney, colon and prostate (dorsal and ventral lobes) were harvested. The tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with

hematoxylin and eosin. Histopathology evaluation was conducted by [b(4)] [b(4)] .

Table 2. Experiment [#]2: Cellular prime and cellular boost in [b(4)] male rats

Group	Rats	Prime (day 0)	Boost (day 14 and day 28)
Control (C)	5	Unimmunized	Unimmunized
Treated (T)	5	spDC + rPAP·rGM-CSF (IV)	spDC + rPAP·rGM-CSF (IV)

Results:

Prostate: Prostate histology was normal for all four unimmunized (Group C) rats. Interstitial prostatitis was evident in 4/4 treated (Group T) rats. One animal had grade 1 lesions, two animals had grade 2 lesions, and one animal had grade 3 lesions. No animal had grade 4 lesions. The prostate of the rat with grade 3 lesions had perivascular accumulation of plasma cells, mononuclear cells and lymphocytes, with neutrophils scattered throughout the interstitium. In some areas of the prostate lymphocytes and neutrophils were marginated in small interstitial vessels and in the periprostatic adventitia.

Non-prostate tissues: In both spDC immunized and unimmunized animals all organs evaluated (brain, lung, heart, liver, kidney and colon) had normal histology, with no tissue infiltration.

Experiment [#]3: Cellular prime, followed by a protein or cellular boosting regimen was designed to investigate the induction of prostatitis, as well as any other microscopic changes, in various non-prostate tissues (Table 3). Half of the [b(4)] male rats (10 weeks old) received primary immunizations of rPAP·rGM-CSF-pulsed cultured spleen cells (rPAP·rGM-CSF-Cx-sp-cells, $5x10^6$ cells/0.5 ml, pulsed with 2 μg/ml of either PAP or OVA), followed by boosting with the same cellular immunization (Group 4) or with fusion protein rPAP·rGM-CSF alone (200 μg/0.5 ml) administered either IV (Group 5) or SC (Group 6). Control rats were immunized with Ova-pulsed cultured spleen cells (Ova-Cx-sp-cells), followed by boosting with either the same pulsed cells (Group 1) or Ova alone by IV (Group 2) or SC (Group 3) injection. Three additional rats ([#]22-24) were injected with saline only (negative controls).

On day 28 (2 weeks after the final boost) the rats were sacrificed and the spleens were removed for use in immunological studies. The brain, heart, lung, thymus, liver, kidney, epididymis, spleen and testes were harvested from Groups 4-6 and from the saline controls and the prostates were harvested from all rats. All tissues were prepared using a routine methodology and stained with hematoxylin and eosin.

Histopathology evaluation was conducted by [b(4)] [b(4)]) for evidence of potential cross-reactive autoimmunity.

Table 3. Experiment [#]3: Cellular prime and protein or cellular boosts in [b(4)] male rats

Group	Rats	Prime (day 0)	Boost (day 7 and day 14)
1	3 (#1-3)	Ova-Cx-sp-cells (IV)	Ova-Cx-sp-cells (IV)
2	3 (#4-6)	Ova-Cx-sp-cells (IV)	200 µg Ova (IV)
3	3 (#7-9)	Ova-Cx-sp-cells (IV)	200 µg Ova (SC)
4	3 (#10-12)	rPAP·rGM-CSF-Cx-sp-cells (IV)	rPAP·rGM-CSF-Cx-sp-cells (IV)
5	3 (#13-15)	rPAP·rGM-CSF-Cx-sp-cells (IV)	200 µg rPAP·rGM-CSF (IV)
6	3 (#16-18)	rPAP·rGM-CSF-Cx-sp-cells (IV)	200 µg rPAP·rGM-CSF (SC)

Results:

Prostate: The prostate pathology in control (Groups 1-3) or saline injected ([#]22-24) animals did not exceed grade 1 inflammation. Mild to severe interstitial inflammation was observed in Groups 4-6 rats. The inflammation was multifocal and consisted of perivascular accumulation of plasma cells, mononuclear cells, and lymphocytes in some areas, with the presence of neutrophils. The inflammation extended into the glandular epithelium and gland lumen. Moderate subacute multifocal interstitial prostatitis was observed in 1/3 Group 4 rats, 3/3 Group 5 rats (grade 2 or higher inflammation), and 2/3 Group 6 rats (grade 3 lesions).

Non-prostate tissues: There were no significant microscopic findings in the brain, heart, liver, spleen, kidneys, colon or testes of any animal. Trace inflammation in the epididymis was observed in 3/3 saline injected animals and mild inflammation in the epididymis was noted in 4/9 rPAP•rGM-CSF-immunized animals. This interstitial inflammation was composed of neutrophils and a few eosinophils. Hemorrhage in the lungs of saline injected animals was considered a result of the euthanasia procedure. The cause of the minimal thymic inflammation (cyst/focal acute inflammation) observed in three animals [one in Group 3 and two in Group 4] was not determined.

Study conclusions: Priming with rPAP•rGM-CSF pulsed APCs, followed by two systemic boosts with rPAP•rGM-CSF or rPAP•rGM-CSF pulsed APCs resulted in a prostate-specific inflammatory immune response; none of the major non-prostate organs showed abnormal microscopic findings, suggesting that the PAP•GM-CSF fusion protein pulsed APCs are capable of breaking immune tolerance in the prostate.

Comment:

- For the various experiments, the rationale for the selection of the tissues for histopathology analysis was not provided in the study report. However, based on the information provided by the sponsor, human PAP is expressed in many other normal tissues, such as bladder, breast, pancreatic islet cells, eyes, head and neck, heart, muscle, peripheral nervous system, liver, salivary gland and skin. Since these tissues were not microscopically examined in all normal tissues expressing PAP antigen, it is not known if any pathology occurred in these PAP-expressing tissues after immunization.

References:

(1). Fong L et al. 1997. Induction of tissue-specific autoimmune prostatitis with prostatic acid phosphatase immunization: implications for immunotherapy of prostate cancer. *J Immunol.* 159:3113-3117.
(2). Peshwa MV et al. 1998. Induction of prostate tumor-specific CD8+ cytotoxic T-lymphocytes in vitro using antigen-presenting cells pulsed with prostatic acid phosphatase peptide. *Prostate.* 36:129-38.
(3). Burch PA et al. 2000. Priming tissue-specific cellular immunity in a phase I trial of autologous dendritic cells for prostate cancer. *Clin Cancer Res.* 6:2175-82.
(4). Laus R et al. 2001. Dendritic cell immunotherapy of prostate cancer: preclinical models and earlier clinical experience. *Cancer Research Therapy and Control.* 11:1-10.

Toxicology Studies

Toxicology studies, as described in the International Conference on Harmonization (ICH) Safety ('S') guidelines, consisting of pharmacokinetics, acute toxicology, chronic toxicology, genotoxicity, carcinogenicity, reproductive and developmental toxicity, safety pharmacology, and immunotoxicity (http://www.ich.org/cache/compo/276-254-1.html) were not conducted due to the autologous nature of Sipuleucel-T and the patient population of focus evaluated in this BLA.

CONCLUSION

Due to the autologous nature of PROVENGE®, limited preclinical studies were conducted in support of this BLA. Pharmacology studies conducted by the sponsor demonstrated that PAP is a potential immune target for prostate cancer active immunotherapy. *In vitro* studies showed that two murine T cell hybridoma cell lines that responded to both murine and human HLA-DR1^{+}APCs and recognized two HLA-DR1 restricted PAP-specific epitopes could be established, indicating that human PAP can be taken up, processed and presented in the context of a human MHC class II molecule.

Immunization of mice or rats with hPAP, hPAP/ b(4) , rPAP or hPAP•mGM-CSF resulted in both humoral and cellular immune responses to PAP antigen. While immunization of mice with rPAP•rGM-CSF could induce prostate-specific inflammation in rats, no

inflammation was observed in a limited set of normal non-prostate tissues examined. Thus, breaking of tolerance to autologous prostate tissue was shown to be possible, providing a rationale to support clinical development of PROVENGE®. Mice immunized with hPAP•hGM-CSF loaded APCs prior to challenge with murine EL4 lymphoma transfected with the human PAP gene showed prolonged survival compared to the control groups; however, the rationale for selection of the dose level of the hPAP•hGM-CSF loaded APCs used, as well as the immunization regimen, was not provided. In this tumor challenge model, immunological endpoints associated with an anti-tumor response mediated by hPAP•hGM-CSF loaded APCs were not determined. In addition, the anti-tumor response in mice bearing established tumors prior to immunization was not evaluated with the rodent equivalent of Sipuleucel-T.

In vitro analysis of PAP protein or PAP gene expression in human tissues demonstrated high expression of the PAP protein or gene in normal and malignant prostate tissue, with significantly lower expression in a limited set of non-prostate normal tissues.

Toxicology studies, as described in the International Conference on Harmonization (ICH) Safety ('S') guidelines, consisting of pharmacokinetics, acute toxicology, chronic toxicology, genotoxicity, carcinogenicity, reproductive and developmental toxicity, safety pharmacology, and immunotoxicity (http://www.ich.org/cache/compo/276-254-1.html) were not conducted due to the autologous nature of Sipuleucel-T and the patient population of focus evaluated in this BLA.

Key Words/Terms: active immunotherapy; asymptomatic metastatic androgen independent prostate cancer; autoimmune prostatitis; immunogenicity; PA2024; PAP•GM-CSF *ex vivo* activated PBMCs or rodent splenocytes; PAP expression; pharmacology; prostate cancer; PROVENGE®; PSA; recombinant hPAP•hGM-CSF; rPAP•rGM-CSF and hPAP•mGM-CSF fusion proteins; Sipuleucel-T; toxicology